ABORTIFACIENT EFFECT OF SALMONELLA ABORTUS-EQUI ENDOTOXIN IN MICE

by

Douglas H. Erbeck

B.S., Kansas State University, 1959
D.V.M., Kansas State University, 1960

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

Approved by:

Stanley M. Davis
Major Professor

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iv
INTRODUCTION	1
I. REVIEW OF LITERATURE	
Endotoxin	3
Properties of Endotoxin	3
Effects of Endotoxin on Pregnancy	4
Pathogenesis of Endotoxin-induced Abortion	5
Tumor Necrosis	7
Prostaglandins	8
Endotoxic Abortion and Normal Parturition	9
Salmonella abortus-equi Infection	10
References	12
II. EFFECT OF SALMONELLA ABORTUS-EQUI ENDOTOXIN IN PREGNANT MICE	
Introduction	16
Materials and Methods	17
Mice	17
Endotoxin	18
Lethal dose$_{50}$	18
Effect of Endotoxin at various stages of Pregnancy	19
Rapidity of Endotoxin Action	20
Lowest Abortifacient dose of Endotoxin	20

Clinical Signs. 21
Temperature Response. 21
Histopathological Examination 21
Results 22
LD_{50}. 22
Effect of S. abortus-equi Endotoxin at
Various Stages of Pregnancy 22
Rapidity of Endotoxin induced Uterine
Changes 23
Lowest aborting dose of S. abortus-equi 25
Clinical Signs. 25
Rectal Temperature Response 27
Histopathological Findings. 27
Discussion. 28
Summary 33
References. 35
Tables. 37
Figures 41
III. OBSERVATIONS ON PREGNANCY DIAGNOSIS IN LABORATORY MICE BASED ON PRESENCE OF VAGINAL PLUGS
Introduction. 44
Materials and Methods 45
Mice. 45
Pregnancy Diagnosis 45
Vaginal Plugs 46

Pups. 46
Results 46
Pregnancy Diagnosis 46
Vaginal Plugs 47
Number of Pups at Term. 48
Discussion. 48
Summary 50
References. 51

APPENDICES

A. LD_{50} of S. abortus-equi endotoxin in mice 53
B. Effect of 150µg S. abortus-equi endotoxin in pregnant mice 54
C. Effect of 50µg S. abortus-equi endotoxin in pregnant mice 63
D. Rapidity of action of S. abortus-equi endotoxin in pregnant mice. 66
E. Lowest abortifacient dose of S. abortus-equi endotoxin in pregnant mice. 69

ABSTRACT

ACKNOWLEDGMENTS

Much of what I have accomplished in life I owe to education. I have been fortunate to claim Kansas State University, College of Veterinary Medicine as my alma mater and now for graduate training. In particular, I would like to thank the Department of Pathology for giving me the opportunity to pursue a lifelong goal.

My major professor, Dr. Stanley Dennis, has given me the opportunity to explore the effects of endotoxin on pregnancy and introduced me to cellular and molecular pathology. Dr. Brad Fenwick accepted me as an additional responsibility and has given much advice on endotoxin and scientific writing. Dr. Jim Cook has nurtured my interest in anatomic pathology and Dr. Jake Mosier has smoothed my transition from practitioner to academician. My wife, Faye, has given me loving and practical support. Everyone with whom I have come in contact in my educational pursuit has contributed to the success of this thesis and I thank each of you.

In life when a man counts his treasures, one of the most valued is that of the friends he has made. I have been blessed by the friendship of Dr. Sam Kruckenberg. This thesis is the first step in my continuing quest for a Ph.D. and Board certification in pathology. I would like

to thank Sam by dedicating this portion of my education to my colleague and friend.

INTRODUCTION

Abortion and infertility are major constraints in the productivity of domestic animals. An identified potent abortifacient is endotoxin from many Gram-negative bacteria. The majority of bacterial abortions in domestic animals are due to Gram-negative organisms. Many undiagnosed abortions may result from endotoxins in systemic or severe localized infections by Gram-negative bacteria. Pregnant animals are reported to be more sensitive to endotoxins.

The effects of Gram-negative endotoxins on pregnancy have received relatively little attention. Diarrhea, fetal death, abortion, shock and maternal death may be induced by endotoxins; the responses are dose-related but interrelationships are complex. Although a number of mechanisms have been proposed, the mechanism involved in endotoxin-induced abortion has not been clarified. Whether the mechanism is the same for all types of abortion, infectious and non-infectious, is not known. It is evident from the literature that endotoxic abortion is different from the other known responses of endotoxin.

Salmonella abortus-equi infection is a specific equine disease and is characterized by abortion, neonatal septicemia, and testicular lesions in stallions. The abortion rate may be as high as 90%. Repeated abortions and birth of infected foals may occur as a result of infection

persisting in the uterus. This research was undertaken to study the effect of S. abortus-equi endotoxin on pregnancy in the laboratory mouse, an accepted model for endotoxin studies, instead of the horse for economic reasons.

The objectives were to study the effects of S. abortus-equi endotoxin in pregnant mice:

1. At various stages of pregnancy;
2. To determine the rapidity of action and lowest abortifacient dose; and
3. To assess the clinical response.

I. REVIEW OF LITERATURE

ENDOTOXIN

Properties of Endotoxin

Endotoxin or lipopolysaccharide (LPS) of Gram-negative bacteria is a macromolecular complex composed of lipid, polysaccharide and protein. It is an integral part of the cell wall and is released on cellular lysis. Lipid A of the endotoxin complex is the biologically active component of endotoxicity (Luderitz et al 1973; Rietschel et al 1982). Although distinctly different from each other antigenically, endotoxins are remarkably similar in the pathophysiological reactions they produce in animals and man (Thomas 1954; Wolff 1973; Goodman et al 1979; Culbertson and Osburn 1980; Rietschel et al 1982). The terms endotoxin and LPS are often used interchangeably.

Endotoxin as released from disrupted bacterial cells in vivo is in a dynamic state of continuous degradation (Westphal 1984). When injected into experimental animals endotoxin produces a variety of effects including fever, diarrhea, hypotension, leukopenia followed by leukocytosis, hyperglycemia, disseminated intravascular coagulation, shock, death, localized and generalized Schwartzman reactions, altered resistance to some bacterial infections and abortion (Thomas 1954; Culbertson and Osburn 1980). Hypothermia and not fever occurs in mice (Zahl and Hutner 1944). Although endotoxins have been of worldwide interest

for over 50 years, many important questions are still unanswered and research continues to increase (Westphal 1984).

Effect of Endotoxin on Pregnancy

Endotoxins are abortifacient in animals and man (Zahl and Bjerknes 1943, 1944; Takeda and Tsuchiya 1953; Rieder and Thomas 1960; McKay and Wong 1963; Dennis 1966; Skarnes and Harper 1972; Miller 1977). Pregnant animals are reported to be more sensitive to endotoxins (Apitz 1935). The majority of bacterial abortions in domestic animals are caused by Gram-negative organisms. Endotoxins may also be responsible for many undiagnosed abortions since any systemic or severe localized infection by Gram-negative bacteria can result in endotoxemia and subsequent abortion in pregnant animals, especially in the last trimester (Dennis 1966, 1980).

Although the effects of endotoxin on pregnancy have been known since 1872, they have received little attention (Dennis 1966, 1987). Endotoxin was reported to be abortifacient in mice and rabbits and the resulting placental changes were similar to endotoxin-induced tumor hemorrhage and necrosis (Zahl and Bjerknes 1943, 1944). Endotoxin-induced clinical signs in increasing severity in pregnant mice are diarrhea, fetal death, abortion, shock and maternal death which appear to be dose-related (Rieder

and Thomas 1960; Dennis 1961, 1966). The interrelationship between these responses are complex.

Pathogenesis of Endotoxin-induced Abortion

Endotoxin-induced abortion has been studied by a number of workers but the pathogenesis is not understood. The effects of endotoxin that have been suggested or incriminated in the pathogenesis include:

1) fever (Zahl and Bjerknes 1944; Takeda and Tsuchiya 1953);
2) placental hemorrhage (Zahl and Bjerknes 1943, 1944; McKay and Wong 1963);
3) altered placental hemodynamics including vascular collapse (Thomas 1954; Persaud 1974);
4) placental Schwartzman reaction (Takeda and Tsuchiya 1953);
5) fetal death (McKay and Wong 1963);
6) serotonin (Dennis 1961; Parant and Chedid 1964);
7) hypersensitivity (Osborne and Smibert 1964; Culbertson and Osburn 1980);
8) altered placental transport (Svihovec et al 1972);
9) changes in newly growing blood vessels (Gasic et al 1975);
10) disseminated intravascular coagulation (Skarnes et al 1981);
11) infarction and anoxia (MacDonald et al 1978);

12) prostaglandin F_{2a} release (Skarnes and Harper 1972; MacDonald et al 1978) and other prostaglandins (Goto et al 1980; Bloch et al 1983);

13) thrombosis of maternal vascular spaces (Manns 1983);

14) trophoblast damage (Manns 1983);

15) placental necrosis; cachectin, a tumor necrosis factor (Zahl and Bjerknes 1943; Beutler and Cerami 1987);

16) alterations of cyclic AMP and cyclic GMP in interactions of catecholamines and/or sex hormones (Shaw and Cameron 1978, 1979);

17) destructive actions of lipoxygenase pathway products (leukotrienes) (Carraher et al 1983);

18) prostaglandin and thromboxane effects on sex hormones (Wilson 1983); and

19) lowering levels of alpha fetoprotein and pregnancy associated murine protein-2 (Hau et al 1987).

Attempts to block endotoxin-induced abortion in mice by various pharmacological drugs and physical means were unsuccessful (Rieder and Thomas 1960; Dennis 1961). Rieder and Thomas (1960) considered abortion a specific endotoxin-induced response, mediated by an undefined mechanism that had certain features in common with endotoxin-induced tumor hemorrhage. Neither maternal lesions (Reider and Thomas 1960) nor fetal death (Parant and Chedid 1964) were considered responsible for triggering endotoxin-induced abortion. Dennis (1961, 1966) concluded that the effect of

endotoxin was not mediated centrally but occurred locally in the uterus to induce abortion through a mediator synthesized in the uterus, possibly 5-hydroxytryptamine (serotonin). It appears from all reports that endotoxin-induced abortion apparently does not share mechanisms with other responses to endotoxin.

Endotoxins will "turn on every defense at our disposal" (Thomas 1954). Responses have been demonstrated to be the result of both direct endotoxin-mediator interactions as well as indirect effects (Morrison and Ulevitch 1978). Abortion appears to be a defensive response of pregnant animals to endotoxin (Dennis 1987).

Tumor Necrosis

Hemorrhagic tumor necrosis resembles a localized Shwartzman-like reaction induced by endotoxin and probably involves endogenous mediators (Freudenberg et al 1984). Zahl and Bjerknes (1943, 1944) and Rieder and Thomas (1960) considered endotoxin-induced placental changes to be similar to tumor necrosis. Clinical interest has recently refocused on the mechanism of action of endotoxin on neoplasms (Westphal 1984) and, a tumor necrosis factor, cachectin, has been identified (Beutler and Cerami 1987). Because of placental changes, cachectin may possibly have a role in endotoxin-induced abortion.

Prostaglandins

Prostaglandin (PGF_{2a}) has been reported to closely simulate the action of endotoxin-induced abortion in mice; the endometrium, not placenta or fetus, is the primary site of synthesis (Skarnes and Harper 1972; Harper and Skarnes 1972). PGF_{2a} may directly act on the uterus by affecting cyclic AMP or indirectly through changes induced in catecholamines and/or sex hormones (Harper and Skarnes 1972; Shaw and Cameron 1978). Intrauterine fetal death, however, appeared to be unrelated to a direct effect of PGF_{2a} on the uterus and was possibly due to other substances, possibly serotonin, released by endotoxin (Skarnes and Harper 1972). PGF_{2a} is a potent stimulator of myometrial contractility.

Several effects attributed to endotoxins including increased vascular permeability, pulmonary smooth muscle contraction and abortion are considered to be induced by prostaglandins or leukotrienes. These substances may represent true mediators that act on susceptible target cells or organs distinct from the reticulo-endothelial system (Schade et al 1984). Prostaglandins and/or leukotrienes are reported to induce abortion (Labhsetwar 1972; Skarnes and Harper 1972; Harper and Skarnes 1972; Schade et al 1984).

Endotoxic Abortion and Normal Parturition

Abortion is a major constraint in animal production and whether abortion and normal parturition are mediated through the same pathway involving prostaglandins, corpus luteum lysis, decrease in progesterone and increase in estrogen levels, and myometrial contractions is unknown. Whether the mechanism is the same for all abortions, infectious and noninfectious, is also unknown (Dennis 1980). PGF_{2a} is involved in luteolysis and parturition in several animal species and man (Liggins et al 1972) and apparently in endotoxin-induced abortion (Skarnes and Harper 1972; Harper and Skarnes 1972). Prostaglandins may play an active role in the immediate responses to endotoxin.

Endotoxin-induced abortion has many of the characteristics of normal parturition. A common factor of normal parturition in all species is a dramatic rise of prostaglandins from uterine tissues (Mitchell 1984). Endotoxins stimulate synthesis of prostaglandins in parenchymal tissues through the cyclooxygenase pathway (Fig. 1).

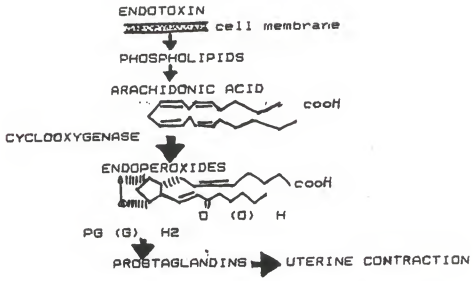


Figure 1 - Biosynthesis of prostaglandins from phosphlolipids following endotoxin injection (Culbertson and Osburn 1980).

Uterine changes associated with endotoxin-induced abortion include the effects of prostaglandins but these effects also occur in normal parturition (Skarnes and Harper 1972; Mitchell 1984; Schade et al 1984).

Salmonella abortus-equi Infection

Salmonella abortus-equi infection is a specific disease of Equidae characterized by abortion, testicular lesions in stallions, and neonatal septicemia (Blood et al 1983). It is present in parts of Europe, South Africa and South America. The disease was common in the United States before 1930 and since 1932 it has almost disappeared.

Abortion occurs between 6 to 9 months gestation, usually between 7 to 8 months. The abortion rate may reach 50 to 90%. Infection may persist in the uterus and cause repeated abortions or infection of future foals. Retained fetal membranes and metritis are common sequelae. Infected foals are born weak and usually die from septicemia within a few days. Surviving foals develop polyarthritis 7 to 14 days later.

The fetal membranes are edematous and hemorrhagic with focal areas of necrosis. The fetus is usually autolytic. S. abortus-equi can be isolated from the fetal membranes, uterine discharge, organs and fluids from the aborted foal, and from joints of foals with polyarthritis.

Clinical signs in affected stallions include fever, edematous swelling of the prepuce and scrotum, arthritis, hydrocele, epididymitis and inflammation of the tunica vaginalis. The scrotal sac lesions are followed by orchitis and testicular atrophy (Blood et al 1983).

REFERENCES

Apitz K: A study of the generalized Shwartzman phenomenon. J Immunol 1935; 29:255-266.

Beutler B, Cerami A: Cachectin: More than a tumor necrosis factor. New Eng J Med 1987 316:379-385.

Bloch MH, McLaughlin LL, Martin SA, Needleman P: Prostaglandin production by the pregnant and nonpregnant rabbit uterus. Prostaglandins 1983;26:33-46.

Blood DC, Radostits OM, Henderson JA: Veterinary Medicine, 6th ed., Bailliere Tindall, London, 1983, pp. 588-589.

Carraher R, Hahn DW, Ritchie DM, McQuire JL: Involvement of lipoxygenase products in myometrial contractions. Prostaglandins 1983;26:23-32.

Culbertson R, Osburn BI: The biological effects of bacterial endotoxin: a short review. Vet Sci Commun 1980;4:3-14.

Dennis SM: Studies on the pathogenesis of Vibrio fetus infection. PhD. Dissertation, University of Sydney, 1961.

Dennis SM: The effect of bacterial endotoxin on pregnancy. Vet Bull 1966;36:123-128.

Dennis SM: Infectious bovine abortion: a practitioners approach to diagnosis. Vet Med/Sm Anim Clin 1980;75:459-466.

Dennis SM: Pregnancy wastage in domestic animals. Kansas State University, Veterinary Medical Center: 1987;289-432.

Freudenberg N, Kensuke J, Galanos C, Freudenberg MA, Westphal O: Hemorrhagic tumor necrosis induced by endotoxin. In: Bacterial Endotoxin Chemical, Biological and Clinical Aspects. Ed. by JY Homma et al, Verlag Chemie, Deerfield Beach, FA, 1984, pp. 261-268.

Gasic GJ, Gasic TB, Strauss JF III: Abortifacient effects of Vibrio cholerae exo-enterotoxin and endotoxin in mice. J Reprod Fert 1975;45:315-322.

Goodman, ML, Way BA, Irwin JW: The inflammatory response to endotoxin. J Pathol 1979;128:7-14.

Goto F, Fujita T, Otani E, Yamamuro M: The effect of indomethacin and adrenergic receptor blocking agents on rats and canine responses to endotoxin. Circ Shock 1980;7:413-424.

Harper MJK, Skarnes RC: Inhibition of abortion and fetal death produced by endotoxin or prostaglandin F_{2a}. Prostaglandins 1972;2:295-309.

Hau J, Basse A, Wolstrup C: A murine model for the assessment of placental and fetal development on teratogenicity studies. Lab Anim 1987;21:26-30.

Labhsetwar AP: Prostaglandin E2: analysis of effects on pregnancy and corpus luteum in hamsters and rats. Acta Endocrin Suppl 1972;170:1-32.

Liggins GC, Grieves S, Kendall TZ, Knox BS: The physiological roles of progesterone, oxestradiol-17 and prostaglandin F_{2a} in the control of ovine parturition. J Reprod Fertil, 1972; Suppl 16:85-100.

Luderitz O, Galanos C, Lehmann V, Nurminen M, Rietschel ET, Rosenfelder G, Simon M, Westphal O: Lipid A: Chemical structure and biological activity. J Infect Dis 1973;128: S17-S29.

MacDonald PC, Porter JC, Schwartz BE, Johnston JM: Initiation of parturition in the human female. Sem Perinat 2 1978;3:273-286.

Manns JG: The use of prostaglandins for regulation of the estrous cycle and as an abortifacient in cattle. Vet Clin N Am L A Pract 1983;5:169-181.

McKay DG, Wong TC: The effect of bacterial endotoxin on the placenta of the rat. Am J Pathol 1963;42:357-377.

Miller R: A summary of some of the pathogenetic mechanisms involved in bovine abortion. Can Vet J 1977;18:77-94.

Mitchell MD: The mechanism(s) of human parturition. J Devel Physiol 1984;6:107-118.

Morrison DC, Ulevitch RJ: The effects of bacterial endotoxins on host mediation systems. Am J Pathol 1978;93:527-601.

Osborne JC, Smibert RM: Vibrio fetus toxin. I. Hypersensitivity and abortifacient action. Cornell Vet 1964;54:561-572.

Parant M, Chedid L: Protective effect of chlorpromazine against endotoxin-induced abortion. Proc Soc Exp Biol Med 1964;116:906-909

Persaud TVM: The effects of prostaglandin F_{2a} on pregnancy and fetal development in mice. Toxicol 1974;2:25-29.

Rieder RF, Thomas L: Studies on the mechanisms involved in the production of abortion by endotoxin. J Immunol 1960;84:189-193.

Rietschel ET, Zahringer U, Wollenweber HW: Bacterial endotoxins: chemical structure and biological activity. Am J Emerg Med 1982;2:60-69.

Schade V, Luderitz H, Rietschel ET: Endotoxin target cells and endogenous mediators: The role of macrophages and arachidonic acid metabolites. In: Bacterial Endotoxin: Chemical Biological and Clinical Aspects. Ed. by JY Homma et al, Verlag Chemie, Deerfield Beach, FA, 1984, pp. 269-279.

Shaw R Jr, Cameron JA: Effect of endotoxin on uterine cyclic AMP in pregnant mice. Res Comm Chem Pathol Pharm 1978;19:225-232.

Shaw R Jr, Cameron JA: Effects of cyclic AMP altering drugs on endotoxin-induced termination of pregnancy. Res Comm Chem Pathol Pharm 1979;24:49-56.

Skarnes RC, Harper MJK: Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. Prostaglandins 1972;1:191-203.

Skarnes RC, Brown SK, Hull SS, McCracken JA: Role of prostaglandin E in the biphasic fever response to endotoxin. J Ex Med 1981;154:1212-1224.

Svihovec J. Stulc J, Friedrich R, Jiricka Z: The effects of some bacterial toxins on placental transport in the rabbit. Toxicon 1972;10:627-633.

Takeda Y, Tsuchiya I: Studies on the pathological changes caused by the injection of the Shwartzman filtrate and the endotoxin into pregnant rabbits. Japan J Exp Med 1953;23:9-16.

Thomas L: The physiological disturbances produced by endotoxins. J Exp Med 1954;98:467-489.

Westphal O: Endotoxin: General Introduction. In: Bacterial Endotoxin: Chemical, Biological and Clinical Aspects. Ed. by JY Homma et al, Verlag Clinic, Deerfield Beach, FA, 1984, pp. 1-8.

Wilson L Jr.: Effects of estradiol and progesterone on uterine prostaglandin levels in the pregnant rat. Prostaglandins 1983;26:47-54.

Wolff S: Biological effects of bacterial endotoxins in man. Infect Dis 128: 1973 Suppl S259-S264.

Zahl PA, Bjerknes C: Induction of decidua-placental hemorrhage in mice by the endotoxins of certain gram-negative bacteria. Proc Soc Exp Biol Med 1943;54:329-332.

Zahl PA, Bjerknes C: Effect of the endotoxin of Shigella paradysenteriae on pregnancy in rabbits. Proc Soc Exp Bio Med 1944;56:153-155.

Zahl PA, Hutner SH: Temperature factors in the action of certain bacterial endotoxins. Proc Soc Exp Biol Med 1944; 56:156-158.

II. EFFECT OF *SALMONELLA ABORTUS-EQUI* ENDOTOXIN IN PREGNANT MICE

INTRODUCTION

Endotoxins are an integral part of the cell walls of Gram-negative bacteria and are released on cellular lysis (Thomas 1954). Although antigenically distinct, their pathophysiological effects are remarkably similar (Thomas 1954; Goodman et al 1979; Culbertson and Osburn 1980; Rietschel et al 1982). When injected into animals they produce a variety of reactions including fever (hypothermia in a few species), diarrhea, hypotension, shock, death and abortion (Zahl and Hutner 1944; Thomas 1954; Dennis 1972; Culbertson and Osburn 1980; Rietschel et al 1982).

Endotoxins are abortifacient in animals and man (Zahl and Bjerknes 1943; Rieder and Thomas 1960; Dennis 1961, 1966). Pregnant animals are reported to be more sensitive to endotoxins (Apitz 1935). The majority of bacterial abortions in domestic animals are caused by Gram-negative organisms. Endotoxins may be responsible for many undiagnosed abortions since any systemic or severe localized infection by Gram-negative bacteria can result in abortion from endotoxemia especially in the last trimester (Dennis 1966, 1980).

Salmonella abortus-equi infection is a specific disease of Equidae characterized by abortion, neonatal septicemia and polyarthritis, and testicular lesions in stallions. Abortions usually occur between 7 and 8 months gestation and infection may persist in the uterus and cause

repeated abortions or infection of future foals. The abortion rate may be as high as 50 to 90% (Blood et al 1983).

Although endotoxin-induced abortion has been studied by a number of workers, the pathogenesis is not understood. A study into the mechanisms of endotoxin-induced abortion was undertaken with S. abortus-equi endotoxin in laboratory mice, an accepted model for endotoxin studies. Reported here are the effects of S. abortus-equi endotoxin at the various stages of pregnancy in mice.

MATERIALS AND METHODS

Mice

White mice (CF/1)[a] and Swiss mixed at Kansas State University were kept in cages with wood shavings in an air conditioned room maintained at 22-23 C. They were fed mouse pellets and water ad lib. The mice were divided into 4 per cage with a mature male for two weeks and checked each morning for presence of copulation plugs (Newton and Newton 1968). Bred mice were transferred to separate cages marked with the date of service until used in the study.

[a]Sasco, Inc., Omaha, NE.

Endotoxin

Endotoxin was purchased[b] and stock solutions were prepared with sterile physiological saline, 2.5 mg/ml, sterilized by filtration[c], and kept at 2-4 C. Stock solutions were diluted with sterile saline for individual injections of endotoxin concentration. All injections were given intraperitoneally (IP) with glass tuberculin syringes.[d]

Lethal $Dose_{50}$

The LD_{50} for S. abortus-equi LPS was determined by the method of Reed and Muench (1938). Forty-eight nonpregnant female, 20 week old, CF/1 mice, randomly divided into 8 groups of 6, were injected IP with decreasing doses of endotoxin from 300 to 160 ug in 0.15 ml sterile saline per group. The mice were observed for 72 hours and survivors were discarded.

---

[b] S. abortus-equi lipopolysaccharide, No. L-1887, phenol extract, chromatographically purified. Sigma Chemical Company, St. Louis, MO.

[c] Gelman disposable filter assembly, 0.45, Scientific Products, Evanston, IL.

[d] Bd-Hypak tuberculin discardit 1cc syringe. Arista, New York, NY.

Effect of Endotoxin at Various Stages of Pregnancy

The effect of S. abortus-equi endotoxin was determined at the following 9 stages of pregnancy in laboratory mice (Green 1966):

Stage 1 - 0 hour, detection of copulation plug considered as the time of fertilization;

Stage 2 - 5 hours, fertilized ova in the oviducts;

Stage 3 - 2.7 to 3 days, ova in morula stage;

Stage 4 - 4 days, blastocyst developing;

Stage 5 - 4.5 days, blastocysts implanting;

Stage 6 - 7.5 days, placenta beginning to form;

Stage 7 - 10 days, mid-gestation, placentas developed and functioning;

Stage 8 - 14-15 days, fetuses well-developed; and

Stage 9 - 16-17 days, last trimester, fetuses near term.

Fifteen bred mice were injected IP at each stage of pregnancy, 10 received 150 µg endotoxin in 0.15 ml sterile saline and 5 controls received 0.15 ml sterile saline.

The mice were kept in cages lined with white absorbent paper towels to facilitate detection of vaginal discharges and abortion. The mice were checked 3 times daily and dead mice were immediately necropsied. Aborting mice were euthanatized with chloroform and necropsied. All other mice were allowed to proceed to term before being euthanatized and necropsied. Fetuses, uteri and ovaries

from representative mice were removed and placed in 10% buffered neutral formalin (BNF).

Rapidity of Endotoxin Action

Forty pregnant mice in the last trimester were divided randomly into 5 groups and injected IP with 150 μg S. abortus equi and euthanatized with chloroform and necropsied immediately to determine whether the fetuses were living or dead at the following times: 15 minutes - 5 mice; 30 minutes - 10 mice; 1 hour - 10 mice; 3 hours - 10 mice; and 5 hours - 5 mice. If abortion occurred before the allotted time, the mice were immediately euthanatized and necropsied. Groups of 5 control mice in the last trimester were injected with 0.15 ml sterile saline and euthanatized and necropsied at the same intervals. Pregnancy was confirmed before injection by abdominal palpation.

Lowest Abortifacient Dose of Endotoxin

Groups of 5 pregnant mice in the last trimester were injected IP with decreasing doses of S. abortus-equi endotoxin beginning with 100 μg and halved until no abortions were observed. The mice were checked 4 times daily at 7:00 AM, Noon, 3:00 PM and 9:00 PM. Twenty pregnant mice, 10 test and 10 control, were injected with 1.0 μg endotoxin or 0.15 ml sterile saline and allowed to

proceed to term. Fetuses, uteri and ovaries were removed and fixed in 10% BNF.

Twenty KSU Swiss Mx mice in late pregnancy were weighed and the μg S. abortus-equi endotoxin per g body weight was calculated.

Clinical Signs

All test and control mice were observed carefully and clinical signs were recorded. Mice were weighed and the endotoxin dose per gram of body weight was calculated.

Temperature Response

Rectal temperatures were taken in 5 groups of pregnant mice during the last trimester with a BD digital thermometer.[e] Six mice were injected IP with 150 μg, 10 with 50 μg and 10 with 1 μg endotoxin, and two control groups of 10, one injected with 0.15 sterile saline and the other no injection. Temperatures were recorded before injection, and at 5 minutes, 15 minutes, 12 hours and 24 hours post injection (PI).

Histopathological examination

Fetuses, uteri and ovaries were removed from representative test and control mice from all studies and fixed in 10% BNF. The tissues were trimmed, processed in

---

[e]Becton, Dickinson and Co., Rutherford, NJ.

an autotechnicon[f], embedded in paraffin, cut at 6 μm, and stained with hematoxylin and eosin (H&E).

Ten mice in the last trimester were injected IP with 1 μg S. abortus-equi endotoxin and tissues after abortion were compared with saline injected controls. Placental and uterine tissues were evaluated for hemorrhage, necrosis and intravascular coagulation on a score of 1 to 5 in ascending order of severity. Uterine lumen and ovaries were evaluated for hemorrhage only.

RESULTS

Lethal $Dose_{50}$

All mice injected with 240 μg or more of S. abortus-equi LPS died (Appendix A, Table 1). Cumulative total of mice dying calculated against cumulative total of mice living determined the LD_{50} in mature female mice to be 208.5 μg.

Effect of S. abortus-equi Endotoxin at Various Stages of Pregnancy

The effects of 150 μg S. abortus-equi on the various stages of pregnancy are given in Appendix B, Tables 2-8. The dose was not sublethal in pregnant mice as 20% died compared to none of the controls. Mortality decreased as

[f]Technicon Instruments Corp., Terrytown, NY.

gestation increased and was confined to the first half. Pregnancy was probably interrupted in 77.8% of the test mice; 33.3% aborted compared to none of the controls and 44.5% were nonpregnant at term compared to 33.3% of the controls. Abortion increased as gestation increased and maternal mortality decreased. All mice aborting in late pregnancy did so in an average of 6.2 hours PI (range 4 to 13 hours). No test mouse injected after 3 days gestation remained pregnant. A higher percentage of control mice than expected, 33.3% were nonpregnant at term.

The results of 50 μg. S. abortus-equi endotoxin are given in Tables 1 and 2 and Appendix C (Tables 9-11). The 50 μg dose was nonlethal in pregnant mice. Pregnancy was interrupted in 91% of the test mice compared to no abortions in controls. Test mice experienced 38.9% abortion and 51.1% were nonpregnant at term. Visible aborted fetuses were recognizable from day 7.5 gestation. Vaginal discharge indicative of pending abortion was not observed. Placental and uterine hemorrhage was observed at necropsy from day 10 gestation. Test mice pregnant at term had an average of 6.4 pups compared to 10.7 in the controls suggesting partial interruption by resorption.

Rapidity of Endotoxin-induced Uterine Changes

The results are tabulated in Table 3 and Appendix D (Tables 12-13). Seventy-five percent of the 40 test mice

had gross uterine lesions within 5 hours PI; 17.5% vaginal hemorrhage and abortion and 57.5% periplacental hemorrhage. The first signs were uterine congestion and periplacental hemorrhage in contrast to the sharp distinct periphery of the discoidal placenta of the controls. The time sequence of the findings in test mice are summarized.

15 minutes	Uterine congestion.
	Early periplacental hemorrhage visible in 2/5.
30 minutes	Periplacental hemorrhage present in 7/10.
1 hour	Vaginal hemorrhage and signs of abortion in 2/10.
	Four had periplacental hemorrhage.
3 hours	One aborted and 1 with vaginal hemorrhage was aborting.
	Eight had periplacental hemorrhage.
	On incision, the placentas were congested.
5 hours	Three aborted and the other 2 had periplacental and uterine hemorrhage.
	Placentas were congested and a few were becoming detached.

There were no gross uterine lesions in the 25 control mice. Two controls, one at 30 minutes and one at 60 minutes IP, voided bloodstained urine but at necropsy, there were no gross lesions and the fetuses were alive.

Lowest Aborting Dose of S. abortus-equi Endotoxin

The effects of decreasing doses of endotoxin in mice in late pregnancy are summarized in Table 4 and Appendix B (Table 14). The lowest dose resulting in abortion was 0.78 µg. The percent of abortions fell as the dose of endotoxin was reduced but the decrease was erratic and not linear. Seven of 10 mice in the histopathology study aborted from 1 ug S. abortus-equi endotoxin injected IP. The average time of abortion was 6.2 hours PI and there was no apparent relationship between dose and time.

The average weight of 20 KSU Swiss Mx mice in late gestation was 65 g (range 56 to 91g), SD 8.46g. Endotoxin dose in µg/g body weight was calculated: 150 µg = 2.31 µg/g; 100 µg = 1.54µg/g; 50 µg = 0.77 µg/g; 25 µg = 0.385 µg/g; 12.5 µg = 0.19 µg/g; 6.25 µg = 0.96 µg/g, 3.125 µg = 0.48 µg/g; 1.56 µg = 0.24 µg/g; 1.0 µg = 0.015 µg/g; 0.78 µg = 0.012 µg/g (lowest aborting dose); and 0.39 µg = 0.006 µg/g.

Clinical Signs

The first clinical signs were tachypnea, restlessness, and biting at legs and abdomen for about 3 to 6 minutes PI. Slight erythema of the nose was evident at about 10 minutes PI. The restlessness was transitory and disappeared within 10 minutes, often within 2 to 3 minutes. Tachypnea persisted throughout the period of endotoxicity in about

25% of the mice while in the remainder, the respiratory rate returned to normal after the first hour. Piloerection was present at 20 to 30 minutes PI, the mice became lethargic and ceased to move around. By 45 minutes soft feces were passed and the haircoat had a rough ruffled appearance. At one hour, the mice were stationary and huddled up usually in a corner of the cage (Fig. 1). Diarrhea with mucus and conjunctivitis with red swollen eyelids were evident. The mice became hypothermic and the temperature returned to normal after 24 hours. They usually aborted in 6.2 hours PI (range 4 to 13 hours).

Typically mice were depressed, huddled in a corner with their eyes closed and coat ruffled, and dehydrated from not drinking or eating for 24 to 36 hours (Fig. 2). Recovery usually started after 24 hours with movement, some grooming, and interest in eating and drinking. The last sign to disappear was piloerection especially around the nape of the neck that usually persisted for 48 to 60 hours PI.

There was essentially no difference in clinical signs at doses above 6.25 µg endotoxin. With 6.25 µg and lower, recovery began as early as 12 hours PI. Transient depression only was observed with 0.39 µg endotoxin.

Rectal Temperature Response

The results are summarized in Fig. 3. Severe hypothermia (-4.7C) was observed in 4 of 6 mice (66.7%) injected with 150 µg S. abortus equi endotoxin and was evident within one hour PI. Signs of shock became evident as the hypothermia approached 24 hours PI. With the lower doses of 50 and 1 µg endotoxin, rectal temperature returned to normal by 24 hours PI. The severely hypothermic mice had muscular tremors and death occurred if hypothermia persisted beyond 24 hours.

Pregnancy was interrupted at the above 3 doses of S. abortus-equi endotoxin.

Histopathological Findings

The endometrium of aborting mice was congested and edematous and had foci of submucosal hemorrhage. The placentas were congested with marked hemorrhage of the placental labyrinth. Hemorrhage was found in one ovary from a mouse that died. No other lesions were observed in ovaries from mice injected with S. abortus-equi endotoxin up to 150 µg.

Seven of 10 mice injected IP with 1 µg S. abortus-equi endotoxin aborted while none of the saline controls aborted. The histopathological findings are illustrated in Fig. 4. The scores for the test mice averaged 22.3 (range 9 to 31) and the controls averaged 25.5 (range 20 to 33).

In a blind study comparing tissues from endotoxic abortion and normal parturition, only 8 of 20 slides were correctly matched.

DISCUSSION

Salmonella abortus-equi endotoxin was deleterious at all stages of pregnancy in laboratory mice, especially from day 10 when the placenta was developed and functioning. The most sensitive period was the last trimester. The closer to term, the more rapid the abortion.

S. abortus-equi endotoxin is potent having an LD_{50} of 208.5 µg. With 150 µg endotoxin, a fifth of the test mice died. The mortality rate decreased as the length of gestation increased and was confined to the first half. With 50 µg (1/4 LD_{50}) pregnancy interruption was unassociated with maternal mortality. The gross signs were periplacental hemorrhage followed by interplacental or uterine hemorrhage, vaginal bleeding and abortion. The average time for abortion was 6.2 hours. The findings are in keeping with observations of another abortifacient organism, Campylobacter fetus (Dennis 1961). The findings from midgestation also agree with observations with the endotoxins of Shigella paradysenteriae, Salmonella typhimurium, and Rhodospirillum nebrum (Zahl and Bjerknes 1943). Rieder and Thomas (1960) found Escherichia coli endotoxin to be abortifacient in mice in late pregnancy but

with a mortality of 41 to 81% depending on dose. This is contrary to the findings with S. abortus-equi endotoxin and those of Zahl and Bjerknes (1943) and Dennis (1961).

Clinical signs were observed within five minutes of S. abortus-equi endotoxin being injected IP, gross changes from 15 minutes, and abortion as early as one hour PI. The clinical signs and rapidity of action of S. abortus-equi endotoxin during the last trimester was similar to those with C. fetus endotoxin (Dennis 1961).

This study demonstrated the potency of S. abortus-equi endotoxin as an abortifacient and how little was required to cause abortion in late pregnant mice, 1/267 LD_{50} or 0.012 µg/g body weight. Phenol-extracted S. abortus-equi endotoxin proved to be a little more potent than phenol-extracted C. fetus endotoxin at 1/240 LD_{50} (Dennis 1961, 1972). Such low doses of endotoxin being abortifacient raises the question of some idiopathic abortions resulting from absorption of endotoxin from Gram-negative intestinal flora. Although the liver is effective in detoxifying endotoxin crossing the intestinal barrier and entering the bloodstream (Homma et al 1984), liver dysfunction may allow sufficient endotoxin to cause abortion especially during the sensitive third trimester. Dennis (1966) commented that abortion may be secondary to any systemic or severe localized Gram-negative bacterial infection.

Vaginal bleeding was not a constant feature with S. abortus-equi endotoxin but was related to the stage of pregnancy and dose. All mice aborting after day 10 gestation had placental hemorrhage and premonitory vaginal bleeding. Vaginal bleeding was a constant sign in the last trimester and the closer to term, the more rapid and profuse the hemorrhage and again, the more rapid and complete the abortion. Vaginal bleeding was observed usually within 4 to 6 hours and up to 24 hours PI. Before day 10 gestation, vaginal bleeding was not common and embryonic and fetal deaths were usually resorbed. The observed bleeding was dark red in contrast to the brighter red just before visible abortion. These observations were similar to earlier reports (Zahl and Bjerknes 1943; Rieder and Thomas 1960; Dennis 1961, 1966).

Histopathological changes in the placenta and endometrium induced by S. abortus-equi endotoxin were difficult to interpret in mice as many of the same changes were present in the saline controls at term, i.e. normal parturition. Hemorrhage in the placental labyrinth was more marked with abortion. Histopathological changes reported in endotoxin-induced abortion are placental hemorrhage, necrosis, acute inflammation, and intravascular coagulation (Zahl and Bjerknes 1943; Dennis 1961; Hall 1973). Similar changes, however, may be observed with normal parturition. Histopathological changes in placentas

of women undergoing spontaneous abortion are very similar to those observed in prostaglandin-induced abortion (Bullard et al 1973). No signs of degeneration, necrosis, hemorrhage or intravascular coagulation were observed in over 100 ovaries from mice injected with S. abortus-equi endotoxin, a finding in keeping with earlier reports (Zahl and Bjerknes 1943; Dennis 1961). The luteolytic effect of endotoxin on the ovary is not detectable microscopically (Harper et al 1978).

Rectal temperatures in mice with various doses of S. abortus-equi endotoxin revealed significant hypothermia only in mice dying from endotoxicosis. All mice with hypothermia beyond 24 hours PI died. This was in contrast to the findings with C. fetus endotoxin as doses as low as 1/24 LD_{50} resulted in rectal temperature dropping 1.6 to 4.1 C in 1/2 to 4 1/2 hours, usually in 1/2 to 2 hours, and returning to normal in 8 to 24 hours; with hyperthermia before hypothermia (34.5C) the mice usually died (Dennis 1961, 1972). The difference was probably due to the times utilized as the temperatures were taken between 15 minutes and 12 hours PI.

The effects of S. abortus-equi endotoxin were more obvious post-implantation because of visible vaginal bleeding. Before implantation, interruption may also occur from endotoxin affecting oviductal motility and early entry into the uterus may be detrimental to the developing

embryos. In rabbits, endotoxin speeds the zygotes through the oviduct (Gasic et al 1975; Harper et al 1978). In rats, however, endotoxin may cause tubular retention and delayed implantation (Labhsetwar 1972). Partial interruption of pregnancy occurs by resorption and mummification and smaller litters being delivered at term (Dennis 1961, 1966). Incomplete abortion and fetal resorption following endotoxin has been reported during the last trimester (Zahl and Bjerknes 1943; Gasic et al 1975; Valenzuela et al 1978). This was also observed with a random sample of control mice averaging litters of 10.7 pups in contrast to mice in the last trimester injected with 1 µg S. abortus-equi endotoxin (1/208 LD_{50}) averaging 6.4 pups per litter.

It is concluded from this study that S. abortus-equi endotoxin resembles other Gram-negative endotoxins by being abortifacient. It was found to be deleterious at all stages of pregnancy in laboratory mice, but especially from midgestation with the most sensitive period being the last trimester. It is a potent abortifacient in mice being effective at 0.78 µg or 1/267 LD_{50}. The clinical signs were typical of those described for other endotoxins in pregnant mice.

SUMMARY

S. abortus-equi endotoxin was tested at the following 9 stages of pregnancy in laboratory mice: 1 - 0 hour, copulation plug detected, time of fertilization; 2 - 5 hours, fertilized ova in oviducts; 3 - 2.7 to 3 days, morulas at the utero-tubal junction; 4 - 4 days, blastocysts developing; 5 - 4.5 days, blastocysts beginning to implant; 6 - 7.5 days, discoidal placentas developing; 7 - 10 days, midgestation, placentas developed and functioning; 8 - 14 to 15 days, beginning of the third trimester, fetuses well-developed; and 9 - 16 to 17 days, fetuses near term. S. abortus-equi endotoxin was deleterious at all stages of pregnancy, especially from midgestation; the most sensitive period being the last trimester. Pregnancy was interrupted in 77.8% of the test mice compared to 33.3% of the controls when 150 μg endotoxin was injected IP; 20% of the test mice died. The mortality decreased as gestation increased and was confined to the first half. With 50 μg, pregnancy was interrupted in 90% of the test mice with no deaths from endotoxicosis. In the control mice 4.2% were nonpregnant at term. Vaginal bleeding from periplacental and uterine hemorrhage was observed after day 10 gestation. Abortion occurred within 1 to 12 hours, average of 6.2 hours PI. The closer to term, the more profuse the vaginal bleeding and the more rapidly abortion occurred.

Clinical signs were observed within 5 minutes, gross changes from 15 minutes, and abortion as early as 1 hour PI. Typically, affected mice had tachypnea, piloerection, hypothermia, diarrhea, conjunctivitis and anorexia. They were lethargic, disinclined to move, huddled up and not drinking. Recovery usually started after 24 hours PI with movement, grooming, drinking and eating. There was essentially no difference in clinical signs at doses above 6.25 μg endotoxin. The lowest aborting dose of S. abortus-equi endotoxin was 1/267 LD_{50} (0.78 μg or 0.012 μg/g body weight). There was no relationship between dose and time of abortion. It was difficult to interpret the endotoxic-induced histopathological changes from those of normal parturition, the major difference being the degree of placental hemorrhage.

REFERENCES

Apitz K: A study of the generalized Shwartzman phenomenon. J Immunol 1935;29:255-266.

Blood DC, Radostits DM, Henderson JA: Veterinary Medicine, 6th ed, Bailliere Tindall, London, 1983; pp. 588-589.

Bullard PD, Herrick CN, Hindle WH, Hale RW, Pion RJ: Histopathologic changes associated with prostaglandin induced abortion. Contracept 1973;7:133-144.

Culbertson R, Osburn BI: The biological effects of bacterial endotoxin: a short review. Vet Sci Commun 1980;4:3-14.

Dennis SM: Studies on the pathogenesis of Vibrio fetus infection. Ph.D. Dissertation, University of Sydney, 1961.

Dennis SM: The effect of bacterial endotoxin on pregnancy. Vet Bull 1966;36:123-128.

Dennis SM: Hypothermia in mice due to Vibrio fetus endotoxin. Cornell Vet 1972;62:296-299

Dennis SM: Infectious bovine abortion: a practitioners approach to diagnosis. Vet Med/Sm Anim Clin 1980;75:459-466.

Gasic GJ, Gasic TB, Strauss JF III: Abortifacient effects of Vibrio cholerae exo-enterotoxin and endotoxin in mice. J Reprod Fert 1975;45:315-322.

Green EL: Biology of the Laboratory Mouse. 2nd Ed, McGraw-Hill, Inc. 1966.

Goodman, ML, Way BA, Irwin JW: The inflammatory response to endotoxin. J Pathol 1979;128:7-14.

Hall GA: Changes in the rat placenta following inoculation with Salmonella dublin. Am J Pathol 1973;72:103-118.

Harper MJ, Valenzuela G, Hodgson BJ: Accelerated ovum transport in rabbits induced by endotoxin. Prostaglandins 1978;15:43-63.

Homma JY, Kanegasaki S, Luderitz O, Shiba T, Westphal O: Bacterial endotoxin: chemical, biological and clinical aspects. Ed. by JY Homma et al, Verlag Chemic, Deerfield Beach, FA, 1984, pp. 307-316.

Labhsetwar AP: Prostaglandin E_{2a}: analysis of effects on pregnancy and corpus luteum in hamsters and rats. Acta Endocrin 1972;Suppl 170:1-32.

Newton N, Newton M: A simple method of obtaining dated pregnancies and observable labors in experimental mice. Am J Obst Gynec 1968;29:871-874.

Reed LJ, Muench H: A simple method of estimating 50% end points. Am J Hyg 1938;27:493-497.

Rieder RF, Thomas L: Studies on the mechanisms involved in the production of abortion by endotoxin. J Immunol 1960;84:189-193.

Rietschel ET, Zahringer U, Wollenweber HW: Bacterial endotoxins: chemical structure and biological activity. Am J Emerg Med 1982;2:60-69.

Thomas L: The physiological disturbances produced by endotoxins. J Exp Med 1954;98:467-489.

Valenzuela G, Harper MJK, Magnaso L: Endotoxin-induced interruption of early pregnancy in the rabbit. J Reprod Fert 1978;54:205-207.

Zahl PA, Bjerknes C: Induction of decidua-placental hemorrhage in mice by endotoxins of certain Gram-negative bacteria. Proc Soc Exp Biol Med 1943;54:329-332.

TABLE 1 - Effect of 50 μg S. Abortus-equi endotoxin injected IP in pregnant mice

Stage of Pregnancy	Time of Injection	No. of Mice	Test Mice					Control Mice					
			Preg.	Non-preg.	Vaginal Hemorrhage	Aborted	Died	No.	Preg.	Non-preg.	Vaginal Hemorrhage	Aborted	Died
1	0 hour*	10	2	8	0	0	0	5	2	3	0	0	0
2	5 hours	10	1	9	0	0	0	5	4	1	0	0	0
3	3 days	10	0	10	0	0	0	5	2	3	0	0	0
4	4 days	10	1	8	0	0	1**	5	3	2	0	0	0
5	4.5 days	10	0	9	1***	0	0	5	4	1	0	0	0
6	7.5 days	10	1	2	0	7	0	5	4	1	0	0	0
7	10 days	10	0	0	0	10	0	5	2	3	0	0	0
8	15 days	10	3	0	0	7	0	5	4	1	0	0	0
9	17 days	10	0	0	0	10	0	5	5	0	0	0	0
	Total	90	8	46	1	34	1**	45	30	15	0	0	0
	%		8.9	51.1	1.1	37.8	1.1		66.7	33.3	0	0	0

* Time of endotoxin injection after copulation plug detected.
** Died from accidental rectal perforation by a thermometer.
*** Pregnancy interrupted.

TABLE 2 – Summary of the effect of 50 μg S. abortus-equi endotoxin in pregnant mice

Stage of Pregnancy	No. of Mice	% Test Mice				No. of Mice	% Control Mice			
		Pregnant	Nonpreg.	Aborted	Died		Pregnant	Nonpreg.	Aborted	Died
First Trimester	50	8	88	2	2	25	60	40	0	0
Midpregnancy	20	5	10	85	0	10	60	40	0	0
Last Trimester	20	15	0	85	0	10	90	10	0	0
Total	90	8.9	51.1	38.9	1.1	45	75	25	0	0

TABLE 3 - Rapidity of S. abortus-equi endotoxin-induced gross lesions in the uterus of mice in late pregnancy.

Time post injection	Vaginal hemorrhage and abortion		Periplacental Hemorrhage		% Gross Lesions
	Test Mice	Controls	Test Mice	Controls	
15 minutes	0/5	0/5	2/5	0/5	40
30 minutes	0/10	0/5*	7/10	0/5	70
60 minutes	2/10	0/5*	4/10	0/5	60
3 hours	2/10	0/5	8/10	0/5	100
5 hours	3/5	0/5	2/5	0/5	100
Total	7/40	0/25	23/40	0/5	--
%	17.5	0	57.5	0	75

* One control voided bloodstained urine.

TABLE 4 - Dose-related abortifacient effect of S. abortus-equi endotoxin in mice in late gestation.

Dose µg	Aborted/injected	%	Time (hours)*
100	4/5	80	4.2
50	5/5	100	6.2
25	4/5	80	8.2
12.5	4/5	80	7.2
6.25	2/5	40	5.5
3.13	4/5	80	9.2
1.56	1/5	20	5.0
0.78	2/5**	20	8.0
0.39	0/5	0	--

* Average time of abortion in group after endotoxin injection.

** One premature birth.

Figure 1 - Typical pregnant mouse one hour after injection with S. abortus-equi endotoxin. It is huddled in a corner, depressed, swollen closed eyelids and has piloerection.

Figure 2 - Typical endotoxin-injected pregnant mouse removed and placed on top of its cage. Note piloerection and rough appearance of haircoat, marked depression, and swollen closed eyelids.

Figure 3 - Rectal temperature of pregnant mice following IP injection of S. abortus-equi endotoxin and control mice.

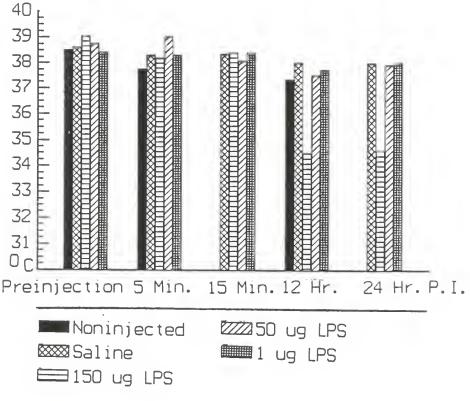
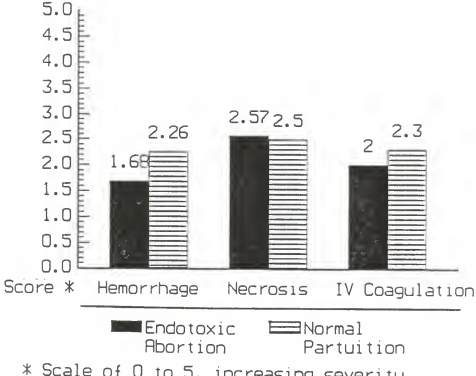

40
39
38
37
36
35
34
33
32
31
0C
Preinjection 5 Min. 15 Min. 12 Hr. 24 Hr. P.I.
Noninjected
50 ug LPS
Saline
1 ug LPS
150 ug LPS

Figure 4 - Microscopic findings in S. abortus-equi endotoxic abortion compared to normal parturition.



* Scale of 0 to 5, increasing severity

III. OBSERVATIONS ON PREGNANCY DIAGNOSIS IN MICE BASED ON PRESENCE OF VAGINAL PLUGS

INTRODUCTION

Laboratory mice mature quickly, are synchronized in estrus by introduction of a male with the majority of females mating on the third night (Short and Woodnutt 1969), have a short gestation with the length of gestation determined from the time the vaginal plug is detected, have a pregnancy rate at term of at least 94%, and are inexpensive for research use (Green 1966; Newton and Newton 1968). Mice are spontaneous ovulators and after copulation and vaginal plug formation, there may be a delay of up to six hours before ovulation and fertilization (Green 1966). Pregnancy diagnosis is based on the presence or absence of vaginal plugs (Zahl and Bjerknes 1943; Green 1966; Newton and Newton 1968) and may be confirmed by abdominal palpation after 10 days (Dennis 1961).

Copulation plugs in mice are used to determine mating and pregnancy. The plugs are formed by a mixture of secretions from the vesicular and coagulation glands of the male and usually fill the vagina from cervix to vulva. Occasionally, smaller less conspicuous plugs are formed. The plugs persist for 16 to 24 hours, occasionally up to 48 hours. The efficiency of copulation plugs for predicting pregnancy is high but strain dependent. The plugs are detected by visual examination in the morning after a dark cycle (Green 1966; Newton and Newton 1968).

Reported here are observations on pregnancy in bred mice based on the presence of vaginal plugs.

MATERIALS AND METHODS

Mice

Eight week mature female KSU Swiss Mx mice weighing 28 g (SD 3.12g), were housed 4 to a cage with a mature male in an air conditioned room at 21 to 23C with a 12 hour dark/light cycle. The males were removed after 2 weeks. The cages were lined with wood shavings and the mice were fed mouse pellets and water ad lib. KSU Swiss Mx mice have a 19 day gestation.

Pregnancy Diagnosis

Initially, the mice were checked each morning for the presence of vaginal plugs. Later, the mice were checked twice daily at the end of the dark and light cycles with a small stainless steel probe and a magnifying loupe for better visibility. Some mice were checked three to four times a day at 6 to 8 hour intervals.

Mice without vaginal plugs were held an additional 2 weeks to determine whether pregnant by abdominal palpation.

Vaginal Plugs

Vaginal precipitations and concretions were examined and vaginal smears were made from sterile cotton swabs and stained by new methyline blue and Giemsa methods, and plugs were fixed in 10% buffered neutral formalin, processed, cut and stained with hematoxylin and eosin (H & E).

Pups

Number of pups at term was compared in pregnant mice surviving S. abortus-equi endotoxins and controls injected with sterile saline.

RESULTS

Pregnancy Diagnosis

Initially of 22 non-injected mice, considered pregnant on presence of vaginal plugs, only 55% were pregnant at term. Later with twice daily examination, 23 of 24 (95.8%) of mice with plugs whelped at term.

Fertility of 76 KSU Swiss Mix mice housed with males for two weeks was 98.7%.

Of 52 evaluated for copulation plugs, 34 (65%) were detectable; 18 (53%) in the morning after 12 hours of darkness and 16 (47%) in the evening after 12 hours of light. The remaining 18 became pregnant without vaginal plugs being detected and were confirmed later by abdominal

palpation. Four of 11 mice (36%) palpated at 10 days gestation were considered pregnant but all were pregnant when palpated on and after day 12 gestation.

Vaginal Plugs

Four distinct types of vaginal plugs were observed; rock-like copulation and three softer pseudo-copulation plugs:

1) Copulation plugs of fertilization were grossly round to ovoid concretions usually present in the vestibule, occasionally extending into the posterior vagina. Microscopically they were composed of irregular amorphous crystals, entrapped spermatozoa, cornified cells but few other recognizable cells (Fig. 1);
2) White putty-like consistency in the vulva and white mucus-like substance lining the vulva;
3) White mucoid crystalline material in the vagina that was easily removed; and
4) White gritty plug in the vulva and extending into the vagina that was easily molded into formed, soft grout-like substance.

The three softer types of vaginal plugs had less distinct morphology and were found in unfertilized female mice.

Number of Pups at Term

A random sample of 10 control mice injected IP with sterile saline had an average litter of 10.7 pups at term compared with an average of 6.4 at term in mice injected with a low dose of S. abortus-equi endotoxin, 1 ug.

DISCUSSION

Twice daily examination at 12 hour intervals for copulation plugs using a magnifying loupe increased the accuracy of pregnancy diagnosis for mice almost two-fold, 55 to 96%. Checking mice more frequently at 6 to 8 hour intervals detected few additional plugs, but the increase was insufficient to justify disturbing the mice and for the time and labor involved. Mice bred better and more frequently when undisturbed for longer periods of time. Movements and activities of the animal caretaker and investigators in the mouse room did not interfere with breeding and fertility in the KSU Swiss Mx mouse colony. It was calculated that less than 10% of the mice randomly distributed to groups would be nonpregnant (SD 0.109).

Bred mice with copulation plugs were found in the morning and the evening. The finding of copulation plugs in the evening after 12 hours of light was contrary to reports that breeding was mainly confined to the dark cycle and

morning examinations were sufficient to detect bred females (Green 1966; Newton and Newton 1968).

More plugs were found when mice were checked at 6 hour intervals but many were not related to fertilization. Spermatozoa within plugs indicated fertilization and they were found only within rock-like plugs. Three other types of vaginal plugs were found in unfertilized females. They were softer, morphologically less distinct, vaginal cells formed a large part of the matrix, and contained no spermatozoa.

Several female mice were examined immediately after observed copulation and many had pseudo-copulation plugs. This suggested that the female produced some of the components of the copulation plug of fertilization being formed. Several other mice were examined after repeated attempts of copulation by the male and most had soft vaginal plugs although they had not been bred.

Pregnant mice are most sensitive to endotoxins during the last trimester and most studies of endotoxin-induced abortion have been confined to this period (Zahl and Bjerknes 1943; Rieder and Thomas 1960; Dennis 1961, 1966). It was concluded from this study that copulation plugs are detectable in approximately two-thirds of mice bred, that pregnancy can occur in the remaining one-third without detectable plugs, and that copulation plugs of fertilization are distinguishable from the softer pseudo-

copulation plugs. Pregnancy is readily confirmed from day 12 gestation by abdominal palpation. Therefore with morning and evening examination for copulation plugs combined with confirmation by abdominal palpation 12 days later it is possible to have 100% pregnant mice of known gestation for use during the last trimester, and at least 90% pregnant mice for the first two trimesters.

SUMMARY

Four distinct types of vaginal plugs were observed. Rock-like copulation plugs of fertilization were distinguishable from the other three types of softer pseudo-copulation plugs unassociated with pregnancy. When morning and evening examinations were conducted for copulation plugs, it was possible to increase pregnancy diagnosis in mice by almost two-fold, 55 to 90%. Copulation plugs were detected in two-thirds of mice bred and pregnancy can occur without detectable vaginal plugs. With bi-daily examination for vaginal plugs, it was possible to provide at least 90% pregnant mice for use during the first two trimesters, and combined with confirmation by abdominal palpation at and after day 12 gestation to provide 100% timed pregnant mice for the last trimester.

REFERENCES

Dennis SM: Studies on the pathogenesis of Vibrio-fetus injection. Ph.D. Dissertation, University of Sydney, 1961.

Dennis SM: The effect of bacterial endotoxin on pregnancy. Vet Bull 1966;36:123-128.

Green EL: Biology of the Laboratory Mouse. 2nd ed, McGraw-Hill, Inc. 1966.

Newton N, Newton M: A simple method of obtaining dated pregnancies and observable labors in experimental mice. Am J Obst Gynec 1968;29:871-874.

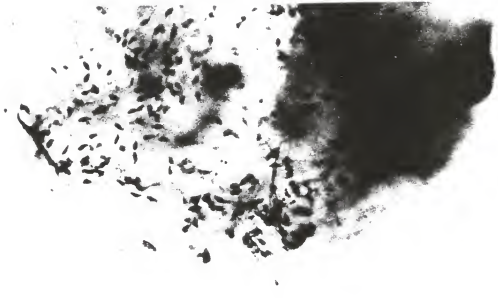
Rieder RF, Thomas L: Studies on the mechanisms involved in the production of abortion by endotoxin. J. Immunol 1960;84:189-193.

Short DJ, Woodnutt DP: The I. A. T. Manual of Laboratory Animal Practice and Techniques. 2nd Ed, Crosby Lockwood & Son, Ltd: 365, 1969.

Skarnes RC, Harper MJK: Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. Prostaglandins 1972;1:191-203.

Zahl PA, Bjerknes C: Induction of decidua-placental hemorrhage in mice by the endotoxins of certain Gram-negative bacteria. Proc Soc Exp Biol Med 1943;54:329-332.

Figure 1 - Photomicrography of a copulation plug of fertilization. Note crystalline-like material enmeshed spermatozoa, and cornified epithelial cells x 100, H & E.



APPENDICES

B. EFFECT OF 150μg *SALMONELLA ABORTUS-EQUI* ENDOTOXIN IN PREGNANT MICE

APPENDIX A

LD_{50} OF S. ABORTUS-EQUI ENDOTOXIN IN MICE

TABLE 1 - Lethality of S. abortus-equi endotoxin injected IP in 20 week-old, nonpregnant mice

Dose (ug)	No. mice dead / No. mice injected	Cumulative Alive	Cumulative Dead	% Mortality
160	0/6	17	0	0
180	0/6	11	0	0
200	4/6	5	4	44
220	3/6	3	7	70
240	6/6	0	13	100
260	6/6	0	19	100
280	6/6	0	25	100
300	6/6	0	31	100

The LD_{50} was between 200 and 220 μg and was calculated to be 208.5 ug by the method of Reed and Muench (1938).

The dose of 150 μg of Salmonella abortus-equi endotoxin was selected from the LD_{50} study as being sublethal and effective for testing at various stages of pregnancy in laboratory white mice. All test and control mice, except those aborting, were allowed to proceed to term and were then euthanatized with chloroform and necropsied. Aborting mice were euthanatized and immediately necropsied. Dead mice were necropsied as soon as they were observed.

The results are given in Tables 2 and 3 and summarized in Table 4. Pregnancy was probably interrupted in 77.8% of the test mice compared to 33.3% of the controls; 33.3% aborted compared to none of the controls, and 44.5% test mice were nonpregnant at term compared to 33.3% of the controls. The findings indicated that mice became more susceptible to the abortifacient effects of S. abortus-equi endotoxin as gestation progressed, especially in the last trimester.

A higher percentage of control mice than expected being nonpregnant at term indicated the need for improving pregnancy diagnosis based on presence or absence of copulation plugs. Pregnancy beyond 10 days was readily confirmed by abdominal palpation.

Twenty percent of the test mice died compared to none of the controls. Mortality decreased as gestation increased and was confined to the first half.

Two conclusions were drawn from this preliminary trial:

1. Select a lower abortifacient dose of S. abortus-equi endotoxin that was independent of maternal lethal effects; and
2. Improve pregnancy diagnosis in mice by studying copulation plugs.

TABLE 2 - Effect of 150µg Salmonella abortus-equi endotoxin* injected intraperitoneally in mice during early pregnancy

Stage of Pregnancy	Time of Injection	Test mice**				Control Mice**			
		Pregnant	Nonpreg.	Vaginal Bleeding	Died	Pregnant	Nonpreg.	Aborted	Died
1	0 hours+	1/10	4/10	2/10	3/10	2/5	3/5	0/5	0/5
2	5 hours	1/10	7/10	0/10	2/10	4/5	1/5	0/5	0/5
3	3 days	0/10	7/10	0/10	3/10	2/5	3/5	0/5	0/5
4	4 days	0/10	4/10	0/10	6/10	3/5	2/5	0/5	0/5
5	4.5 days	0/10	5/10	3/10	2/10	4/5	1/5	0/5	0/5
	TOTAL	2/50	27/50	5/50	16/50	15/25	10/25	0/25	0/25
		4%	54%	10%	32%	60%	40%	0%	0%

* Sigma Chemical Company, St. Louis, MO 63178.
** 150µg endotoxin in 0.15ml sterile saline and controls 0.15ml sterile saline.
\+ Hours of injection after detecting copulation plug.

TABLE 3 - Effect of 150μg Salmonella abortus-equi endotoxin* injected intraperitoneally in mice during mid and late pregnancy.

Stage of Pregnancy	Time of Injection	Test mice**				Control Mice**			
		Pregnant	Nonpreg.	Aborted	Died	Pregnant	Nonpreg.	Aborted	Died
6	7.5 days+	0/10	6/10	3/10	1/10	4/5	1/5	0/5	0/5
7	10 days++	0/10	7/10	2/10	1/10	2/5	3/5	0/5	0/5
8	15 days	0/10	0/10	10/10	0/10	4/5	1/5	0/5	0/5
9	17 days	0/10	0/10	10/10	0/10	5/5	0/5	0/5	0/5
TOTAL		0/40	13/40	25/40	2/40	15/20	5/20	0/5	0/5
		0%	32.5%	62.5%	5%	75%	25%	0%	0%

* Sigma Chemical Company, St. Louis, MO 63178.
** 150μg endotoxin in 0.15ml sterile saline and controls 0.15ml sterile saline.
\+ Time of injection after detecting copulation plug.
++ After 10 days, pregnancy was confirmed by abdominal palpation.

TABLE 4 - Summary of the effect of 150µg Salmonella abortus-equi endotoxin on pregnancy in mice.

Stage of Pregnancy	% Test Mice				% Control Mice			
	Pregnant	Nonpreg.	Aborted	Died	Pregnant	Nonpreg.	Aborted	Died
First Trimester	4	54	10	32	60	40	0	0
Midpregnancy	0	65	25	10	60	40	0	0
Last Trimester	0	0	100	0	90	10	0	0
TOTAL %	2.2	44.5	33.3	20.0	66.7	33.3	0	0

TABLE 5 - Clinical and necropsy findings in 40 mice in the first trimester of pregnancy injected with 150µg S. abortus-equi endotoxin IP.

Mice	Test Mice: Pregnant F O M B	Aborted F O M B	Nonpreg. F O M B	Dead F O M B	Time Hr. F	O	M	B	Mean	SD	%
1	- - - -	1 - - -	- - 1 -	0 1 - 1	36	4		44	0.05	0.217944	5.00 Pregnant
2	- - - -	- - - -	1 1 1 -	- - - 1				24			
3	1 1 - -	- - - -	- - 1 -	- - - 1				24	0.05	0.217944	5.00 Aborted
4	- - - -	- - - -	1 1 1 1	- - - -							
5	- - - -	- - - -	- 1 1 1	1 - - -	36				0.55	0.497493	55.00 Nonpreg.
6	- - - -	- - - -	1 1 - -	- - 1 1			36	36			
7	- - - -	- - - -	- - - -	1 1 1 1	18	28	41	55	0.35	0.476969	35.00 Dead
8	- - - -	- - - -	1 1 - -	- - 1 1			26	36			
9	- - - -	1 - - -	1 1 1	- - - -				60			
10	- - - -	- - - -	- 1 1 1	1 - - -	40						
Total	2	2*	22	14**							

F = Fertilization
O = Oviduct
M = Monula
B = Blastocyst

SD = Standard deviation

* Both had vaginal hemorrhage
** Average time of death, 36 hours PI

TABLE 6 Individual clinical and necropsy findings in 30 mice in the second trimester of pregnancy injected with 150µg S. abortus-equi endotoxin IP.

Test Mice												
Pregnant			Aborted			Nonpreg.			Dead			Time Hr.			Mean	SD	%	
I	P	M	I	P	M	I	P	M	I	P	M	I	P	M				
-	-	-	-	1	-	1	-	1	-	+	-		48		0	0	0.00	Pregnant
-	-	-	-	-	1	-	1	-	1	-	-	37		10				
-	-	-	1	-	-	-	1	1	-	-	-	15			0.266666	0.442216	26.67	Aborted
-	-	-	-	-	-	1	1	1	-	-	-							
-	-	-	-	-	-	-	1	-	1	-	1	24		38	0.6	0.489897	60.00	Nonpreg
-	-	-	-	-	1	1	1	-	-	-	-			5				
-	-	-	-	-	-	1	-	1	-	1	-		24		0.133333	0.339934	13.33	Dead
-	-	-	-	1	-	1	-	1	-	-	-		30					
-	-	-	1	1	-	-	-	1	-	-	-	12	5					
-	-	-	1	-	-	-	1	1	+	-	-	24						
TOTAL		0			8*			18**			4							

I = Implantation
P = Placenta
M = Midpregnancy

SD = Standard deviation

* Two died
** Six died
(Average time, 32.5 hours PI)

TABLE 7 Individual clinical and necropsy finding in mice in the last trimester injected IP with 150µg S. abortus-equi endotoxin.

Test Mice										
Pregnant		Aborted		Nonpreg.		Dead		Time Hr.		Mean	SD	%
D	N	D	N	D	N	D	N	D	N			
-	-	1	1	-	-	-	-	5	5	0	0	0.00 Pregnant
-	-	1	1	-	-	-	-	5	5			
-	-	1	1	-	-	-	-	5	5	1	0	100.00 Aborted
-	-	1	1	-	-	-	-	5	12			
-	-	1	1	-	-	-	+	5	12	0	0	0.00 Nonpreg.
-	-	1	1	-	-	-	-	5	2			
-	-	1	1	-	-	-	-	4	6	0	0	0.00 Dead
-	-	1	1	-	-	-	-	6	4			
-	-	1	1	-	-	-	-	6	6			
-	-	1	1	-	-	-	-	6	13			
TOTAL	0		20*		0		1					

D = Developed fetuses
N = Near term fetuses

*Average time of abortion, 6.1 hours PI

+ Died 12 hours PI

TABLE 8 Control mice of the 150µg S. abortus-equi study injected with 0.15ml sterile saline IP

	Pregnant		Nonpregnant		
	F O M B I	P M D N	F O M B I	P M D N	% Pregnant
	- 1 1 1 1	- 1 1 1	1 - - - -	1 - - -	For all stages 66.67
	1 1 - - 1	1 1 1 1	- - 1 1 -	- - - -	
	- 1 1 - -	1 + 1 1	1 - - 1 1	- 1 - -	
	- 1 - 1 1	1 - - 1	1 - 1 - -	- 1 1 -	For F - M 60.00
	1 - - 1 1	1 - 1 1	- 1 1 - -	- 1 - -	
TOTAL	15	15	10	5	

F = Fertilization
O = Oviduct
M = Morula
B = Blastocyst
I = Implantation

P = Placenta
M = Midpregnancy
D = Developed fetuses
N = Near term fetuses

At the end of this first study, 22 mice with positive plugs were held to term; only 12 actually whelped. Only 54.54% of the mice were pregnant through the entire gestation.

C. EFFECT OF 50μg S. ABORTUS-EQUI ENDOTOXIN IN PREGNANT MICE

TABLE 9 Individual clinical and necropsy findings in mice injected with 50µg <u>S. abortus-equi</u> endotoxin IP during the first trimester of pregnancy.

	Test Mice							
	Pregnant F O M B	Aborted F O M B	Nonpreg. F O M B	Dead F O M B	Time Hr. F O M B	Mean	SD	%
	- - - -	- - - -	1 1 1 1	- - - -		0.1	0.3	10.00 Pregnant
	- - - -	- - - -	1 1 1 1	- - - -				
	- 1 - -	- - - -	1 - 1 1	- - - -		0	0	0.00 Aborted
	- - - -	- - - -	1 1 1 1	- - - -				
	- - - 1	- - - -	1 1 1 -	- - - -		0.875	0.330718	87.50 Nonpreg.
	- - - -	- - - -	1 1 1 1	- - - -				
	1 - - -	- - - -	- 1 1 -	- - - 1	19	0.025	0.156124	2.50 Dead
	1 - - -	- - - -	- 1 1 1	- - - -				
	- - - -	- - - -	1 1 1 1	- - - -				
	- - - -	- - - -	1 1 1 1	- - - -				
TOTAL	4	0	35	1				

TABLE 10 Individual clinical and necropsy findings in mice injected IP with 50µg S. abortus-equi endotoxin during the second trimester of pregnancy.

Test Mice												
Pregnant			Aborted			Nonpreg.			Dead			Time Hr.			Mean	SD	%	
I	P	M	I	P	M	I	P	M	I	P	M	I	P	M				
-	-	-	-	1	1	1	-	-	-	-	-		12	12	0.033333	0.179505	3.33	Pregnant
-	-	-	-	-	1	1	1	-	-	-	-			10				
-	-	-	-	1	1	1	-	-	-	-	-		12	10	0.6	0.489897	60.00	Aborted
-	-	-	-	1	1	1	-	-	-	-	-		12	11				
-	-	-	1	1	1	-	-	-	-	-	-	12	12	10	0.366666	0.481894	37.67	Nonpreg.
-	-	-	-	1	1	1	-	-	-	-	-		12	10				
-	-	-	-	-	1	1	1	-	-	-	-			12	0	0	0.00	Dead
-	1	-	-	-	1	1	-	-	-	-	-			11				
-	-	-	-	1	1	1	-	-	-	-	-		12	11				
-	-	-	-	1	1	1	-	-	-	-	-		12	12				
TOTAL		1			18			11			0							

I = Implantation
P = Placenta
M = Midpregnancy

TABLE 11 Individual findings in mice during the last trimester injected IP with 50μg S. abortus-equi endotoxin.

Test Mice										
Pregnant		Aborted		Nonpreg.		Dead		Time Hr.		Controls		Mean	SD	%
D	N	D	N	D	N	D	N	D	N	Preg.	Nonpreg.			
-	-	1	1	-	-	-	-	5	9	2	-	0.15	0.357071	15.00 Pregnant
-	-	1	1	-	-	-	-	5	22	3	-			
1	-	-	1	-	-	-	-		6	2	1	0.85	0.357071	85.00 Aborted
-	-	1	1	-	-	-	-	14	22	2	-			
-	-	1	1	-	-	-	-	4	4	3	-	0	0	0.00 Nonpreg.
1	-	-	1	-	-	-	-		7	2	-			
-	-	1	1	-	-	-	-	5	8	2	-	0	0	0.00 Dead
-	-	1	1	-	-	-	-	13	24	3	-			
1	-	-	1	-	-	-	-		24	2	-			
-	-	1	1	-	-	-	-	9	5	2	-			
TOTAL 3		17		0		0				23*	1			

D = Developed fetuses
N = Near-term fetuses

*24 mice with copulation plugs served as controls and proceeded to term, 95.83% pregnant at term.

D. RAPIDITY OF ACTION OF *S. ABORTUS-EQUI* ENDOTOXIN IN PREGNANT MICE

TABLE 12 Rapidity of action of 150µg S. abortus-equi endotoxin injected IP in 40 mice in the last trimester of pregnancy.

Mouse No.	Gross Lesions - 15 minutes			Gross Lesions - 30 minutes			Gross Lesions - 1 hour		
	Hemorrhage H V P	Abortion	Nil	Hemorrhage H V P	Abortion	Nil	Hemorrhage H V P	Abortion	Nil
1 Test	- - -	-	1	1 - 1	-	-	1 1 -	-	-
Control	- - -	-	1	- - -	-	1	- - -	-	1
2 Test	- - -	-	1	- - -	-	1	1 - 1	-	-
Control	- - -	-	1	- - -	-	1	- - -	-	1
3 Test	1 - 1	-	-	1 - 1	-	-	- - -	-	1
Control	- - -	-	1	- - -	-	1	- - -	-	1
4 Test	- - -	-	1	1 - 1	-	-	- - -	-	1
Control	- - -	-	1	- - -	-	1	- - -	-	1
5 Test	1 - 1	-	-	- - -	-	1	- - -	-	1
Control	- - -	-	1	1 1 -	-	-	1 1 -	-	-
6 Test				1 - 1	-	-	1 - 1	-	-
7 Test				1 - 1	-	-	1 - 1	-	-
8 Test				- - -	-	1	1 - 1	-	-
9 Test				1 - 1	-	-	1 1 -	-	-
10 Test				1 - 1	-	-	- - -	-	1

TABLE 12 (Continued)

Mouse No.	Gross Lesions - 3 hours			Gross Lesions - 5 hours		
	Hemorrhage H V P	Abortion	Nil	Hemorrhage H V P	Abortion	Nil
1 Test	1 - 1	-	-	1 1 -	1	-
Control	- - -	-	1	- - -	-	1
2 Test	1 - 1	-	-	1 - 1	-	-
Control	- - -	-	1	- - -	-	1
3 Test	1 - 1	-	-	1 - 1	-	-
Control	- - -	-	1	- - -	-	1
4 Test	1 - 1	-	-	1 1 -	1	-
Control	- - -	-	1	- - -	-	1
5 Test	- - -	-	1	1 1 -	1	-
Control	- - -	-	1	- - -	-	1
6 Test	1 - 1	-	-			
7 Test	1 1 -	1	-			
8 Test	1 - 1	-	-			
9 Test	1 - 1	-	-			
10 Test	1 - 1	-	-			

H = Hemorrhage present
V = Vaginal hemorrhage
P = Placental hemorrhage

1 = Positive
- = Negative

TABLE 13 Summary of results of the rapidity of action of S. abortus-equi endotoxin in 40 late pregnant mice.

	Test Mice						Control Mice		
		Hemorrhage					Vaginal		
Time PI	No.	Present	Vaginal	Placental	Aborted	NGL	No.	hemorrhage	Aborted	NGL
15 minutes	5	40%	-	40%	-	60%	5	-	-	100%
30 minutes	10	70%	-	70%	-	30%	5	-*	-	100%
1 hour	10	60%	20%	40%	-	40%	5	-*	-	100%
3 hours	10	90%	10%	90%	10%	10%	5	-	-	100%
5 hours	5	100%	60%	40%	60%	-	5	-	-	100%

NGL = No gross lesions
- = nil percent

* 1 mouse at 3 minutes and 1 at 1 hour PI voided blood-stained urine but pregnant with live fetuses at necropsy and no gross lesions.

E. LOWEST ABORTIFACIENT DOSE OF *S. ABORTUS-EQUI* ENDOTOXIN IN PREGNANT MICE

TABLE 14 - Lowest abortifacient dose of S. abortus-equi endotoxin injected IP into mice during late pregnancy.

Mouse No.	100µg Aborted	Time PI (hours)	50µg Aborted	Time PI (hours)	25µg Aborted	TimePI (hours
1	1	2	1	4	1	12
2	1	6	1	2	1	5
3	-	-	1	7	1	13
4	1	4	1	5	-	-
5	1	5	1	13	1	3
Ave.	80.0%	4.2	100.0%	6.2	80.0%	8.2

	12.5µg Aborted		6.25µg Aborted		3.125µg Aborted	
1	1	11	-	-	1	10
2	1	6	-	-	1	9
3	1	6	-	-	1	11
4	1	6	1	5.5	-	-
5	-	-	1	5.5	1	7
Ave.	80.0%	7.2	40.0%	5.5	80.0%	9.2

	1.06µg Aborted		0.78µg* Aborted		0.39µg Aborted	
1	-	-	Premature	-	-	-
2	-	-	1	8	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	1	4	-	-	-	-
Ave.	20.0%	4	40.0%	8	0.0%	-

* 1/267 LD_{50}

Abortions occurred within 4 to 9.2 hours PI, a mean of 6.2 hours. Time of abortion was not related to dose of S. abortus-equi endotoxin.

ABORTIFACIENT EFFECT OF SALMONELLA ABORTUS-EQUI ENDOTOXIN IN MICE

by

Douglas H. Erbeck

B.S., Kansas State University, 1959
D.V.M., Kansas State University, 1960

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

The abortifacient effect of Salmonella abortus-equi endotoxin was evaluated at the following 9 stages of pregnancy in laboratory mice: 1. - 0 hour, copulation plug detected, time of fertilization; 2. - 5 hours, fertilized ova in oviducts; 3. - 2.7 to 3 days, morulas at the utero-tubal junction; 4. - 4 days, blastocysts developing; 5. - 4.5 days, blastocysts beginning to implant; 6. - 7.5 days, discoidal placentas developing; 7. - 10 days, mid-gestation, placentas developed and functioning; 8. - 14 to 15 days, start of the third trimester, fetuses well-developed; and 9. - 16 to 17 days, fetuses near term. S. abortus-equi endotoxin was deleterious at all stages of pregnancy, especially from mid-gestation; the most sensitive period being the last trimester. With 150µg endotoxin injected intraperitoneally (IP), pregnancy was interrupted in 77.8% of the test mice compared to 33.3% of the controls. Twenty percent of the test mice died; mortality decreased as gestation increased and was confined to the first half. With 50µg, pregnancy was interrupted in 90% of the test mice with no maternal deaths from endotoxicosis. In the control mice 4.2% were nonpregnant at term. Vaginal bleeding from periplacental and uterine hemorrhage was observed from day 10 gestation. Abortion occurred within 1 to 12 hours postinjection (PI), average of 6.2 hours. The closer to term, the more profuse the vaginal bleeding and the more rapidly abortion occurred.

Clinical signs were observed within five minutes, gross changes from 15 minutes, and abortion as early as one hour PI. Typically, affected mice had tachypnea, piloerection, hypothermia, diarrhea, conjunctivitis and anorexia. They were lethargic, disinclined to move, huddled up and not drinking. Recovery usually began after 24 hours with movement, grooming, drinking and eating. The last sign to disappear was piloerection. There was essentially no difference in clinical signs observed at doses above 6.25ug endotoxin. The lowest aborting dose of S. abortus-equi endotoxin was 1/267 LD_{50} (0.78µg or 0.012µg/g body weight). There was no relationship between dose and time of abortion. It was difficult to interpret the endotoxic-induced histopathological changes from those of normal parturition, the major difference being the degree of placental hemorrhage.

Observations on vaginal plugs revealed four distinct types. Rock-like copulation plugs of fertilization were distinguishable from the other three types of softer pseudo-copulation plugs unassociated with pregnancy. With morning and evening examination for copulation plugs in the vulvovagina, it was possible to increase pregnancy diagnosis in mice by almost two-fold, 55 to 96%. Copulation plugs were detected in two-thirds of mice bred and pregnancy can occur without detectable vaginal plugs. With bi-daily examination for vaginal plugs, it was possible to provide at least 90%

pregnant mice for use during the first two trimesters, and combined with confirmation by abdominal palpation at and after day 12 gestation to provide 100% timed pregnant mice for the last trimester.